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(54) Title: USE OF OXALATE DEFICIENT *ASPERGILLUS NIGER* STRAINS FOR PRODUCING A POLYPEPTIDE

(57) Abstract: The invention relates to oxalate deficient *A. niger* strains for the production of a given enzyme, wherein the oxalate deficient strain produces at least the same amount of enzyme as the wild type strain it originates from under the same culture conditions. Preferably, the oxalate deficient *A. niger* strain produces more enzyme than the wild type strain it originates from under the same culture conditions. More preferably, the oxalate deficient *A. niger* strain is such that when the strain has been transformed with an expression construct comprising a gene coding for an enzyme, said strain produces at least the amount of the enzyme the wild type strain it originates from would produce under the same culture conditions, when the wild type strain has also been transformed with the same expression construct as the oxalate deficient strain. The invention also relates to method for obtaining such oxalate deficient *A. niger* strain. The present invention further relates to method for producing an enzyme, wherein an oxalate deficient *A. niger* strain that produces at least the same amount of enzyme as the wild type strain it originates from under the same culture conditions is used.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, MEDLINE, WPI Data, PAJ, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/50576 A (NOVONORDISK AS) 31 August 2000 (2000-08-31) cited in the application Abstract; p. 1, l. 11-15; p. 19, l. 27; p. 23, l. 1-18; claims 24-26.	1-11
X	RUIJTER G J G ET AL: "OXALIC ACID PRODUCTION BY ASPERGILLUS NIGER: AN OXALATE-NON-PRODUCING MUTANT PRODUCES CITRIC ACID AT PH 5 AND IN THE PRESENCE OF MANGANESE" MICROBIOLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 145, no. 9, September 1999 (1999-09), pages 2569-2576, XP000905457 ISSN: 1350-0872 Abstract; p. 2570, col. 2, l. 4-8; Table 2.	1-11

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOMBERGH VAN DEN J P T W ET AL: "NEW PROTEASE MUTANTS IN ASPERGILLUS NIGER RESULTS IN STRONGLY REDUCED IN VITRO DEGRADATION OF TARGET PROTEINS; GENETICAL AND BIOCHEMICAL CHARACTERIZATION OF SEVEN COMPLEMENTATION GROUPS" CURRENT GENETICS, NEW YORK, NY, US, vol. 28, no. 4, 1995, pages 299-308, XP000867320 ISSN: 0172-8083 Abstract; p. 300, col. 1, "Mutagenesis and mutant selection"; Table 2 and Table 4.	1-11
X	WO 97/10350 A (LIKOSPOL S R O ; MINARIK MARTIN (SK); SKVARENINA DUSAN (SK); MICHAL) 20 March 1997 (1997-03-20) Abstract; p. 13, l. 1-14: p. 14, l. 13-16.	1-11
X	PEDERSEN H ET AL: "Construction and characterization of an oxalic acid nonproducing strain of Aspergillus niger." METABOLIC ENGINEERING. UNITED STATES JAN 2000, vol. 2, no. 1, January 2000 (2000-01), pages 34-41, XP002286173 ISSN: 1096-7176 cited in the application Abstract.	1-8
Y	US 6 432 672 B1 (BOVENBERG ROELOF ARY LANS ET AL) 13 August 2002 (2002-08-13) Abstract; col. 26, l. 15-45.	1-11
Y	US 4 518 697 A (BARTNIK FRIEDHELM ET AL) 21 May 1985 (1985-05-21) Abstract; col. 1, l. 30-53	1-11
Y	SARANGBIN S ET AL: "Yam bean starch: a novel substrate for citric acid production by the protease-negative mutant strain of Aspergillus niger" CARBOHYDRATE POLYMERS, APPLIED SCIENCE PUBLISHERS, LTD. BARKING, GB, vol. 38, no. 3, March 1999 (1999-03), pages 219-224, XP004154847 ISSN: 0144-8617 Abstract; p. 220, "Material and methods"; p. 221, "Selection of protease-negative mutant strains with enhanced glucoamylase and acid production".	1-11

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2004/001173

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0050576 A	31-08-2000	AU 2658200 A CN 1341149 T WO 0050576 A1 EP 1157100 A1 JP 2002536993 A US 2003148464 A1 US 6544765 B1	14-09-2000 20-03-2002 31-08-2000 28-11-2001 05-11-2002 07-08-2003 08-04-2003
WO 9710350 A	20-03-1997	SK 113195 A3 SK 15696 A3 AU 7006196 A WO 9710350 A1	07-05-1997 10-09-1997 01-04-1997 20-03-1997
US 6432672 B1	13-08-2002	AU 7642298 A BR 9808859 A CN 1257546 T WO 9846772 A2 EP 0979294 A2 JP 2001518798 T PL 336345 A1	11-11-1998 01-08-2000 21-06-2000 22-10-1998 16-02-2000 16-10-2001 19-06-2000
US 4518697 A	21-05-1985	DE 3149457 A1 DK 516882 A EP 0082395 A2 JP 58107177 A	23-06-1983 15-06-1983 29-06-1983 25-06-1983